PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Artcle 36 and Rule 70)

Applicant's or agent's file reference OP020077	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. **PCT/KR2001/002304**	International filing date *(day/month/year)* 29 DECEMBER 2001 (29.12.2001)	Priority date *(day/month/year)*

International Patent Classification (IPC) or national classification and IPC

IPC7 C12N 5/16

Applicant

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1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demamd	Date of completion of this report
29 JULY 2003 (29.07.2003)	13 APRIL 2004 (13.04.2004)

Name and mailing address of the IPEA/KR

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

national aplication No.
PCT/KR2001/002304

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-30 , as originally filed
pages _____ , filed with the demand
pages _____, filed with the letter of _____

☒ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statment) under Article 19
pages _____ , filed with the demand
pages 31-33 , filed with the letter of 16/03/2004

☒ the drawings:
pages 1/9-9/9 , as originally filed
pages _____ , filed with the demand
pages _____, filed with the letter of _____

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language English which is

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☒ the language of publication of the international application(under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide** and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained inthe international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disc losure in the international applicationas as filed has been furinshed.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☒ the claims, Nos. 14, 15

☐ the drawings, sheet _____

5.

☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box(Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed." and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION

national aplication No.
PCT/KR2001/002304

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-13	YES
	Claims	None	NO
Inventive step (IS)	Claims	1-13	YES
	Claims	None	NO
Industrial applicability (IA)	Claims	1-13	YES
	Claims	None	NO

2. Citations and explanations (Rule 70.7)

The following documents have been considered for the purpose of this report:

D1 = Anim. Reprod. Sci. Vol. 68(1-2): 111-120 (31 October 2001)
D2 = Mol. Reprod. Dev. 57: 331-337 (2000)
D3 = US 6258998A (10 July 2001)

1. Novelty

The present invention relates to a cloned pig with a specific genetic character and to a method of producing such a pig by transfection of desired genes into somatic cells and by somatic cell nuclear transfer. Specifically, claim 1 relates to a method of producing a cloned pig expressing green fluorescent protein by transfecting pEGFP-N1 into a cell and transferring the nucleus of a transfected cell into a recipient oocyte.

D1 discloses porcine embryos derived from nuclear transfer of granulosa-derived cells transfected by a retroviral vector carrying an EGFP gene. D2 discloses porcine embryos produced from nuclear transfer of porcine fetal fibroblasts transfected by retrovirus vector pLNbeta-EGFP. None of the prior art documents disclose transfection using pEGFP-N1 and production of live offspring from porcine embryos derived from somatic cell nuclear transfer. Therefore, the present invention is considered to be novel (PCT Article 33(2)).

2. Inventive step

Claims 1-3 relate to a method of producing a cloned pig expressing green fluorescent protein by transfecting pEGFP-N1 into a cell and somatic cell nuclear transfer of such a transfected cell as a nuclear donor cell.
(Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of:

BoxV

D1 and D2 disclose porcine embryos produced from nuclear transfer of granulosa-derived cells or porcine fetal fibroblasts transfected by a retroviral vector carrying an EGFP gene. D3 discloses method of producing a cloned pig by somatic cell nuclear transfer. A major difference between the prior art (D1, D2, D3) and the present invention is the method of introducing an EGFP gene into a cell. The retroviral vector is used in D1 and D2, while the non-viral vector pEGFP-N1 is used in the present invention. It appears non-obvious for the skilled person in the art to replace retroviral vector into non-viral vector pEGFP-N1 without many experiments. Therefore, the subject-matter of claims 1-3 is considered to involve an inventive step (PCT Article 33(3)).

3. Industrial applicability

The subject matter of claims 1-13 is considered to be industrially applicable (PCT Article 33(4)).

New Citation
Mol. Reprod. Dev. 57: 331-337 (2000)

CLAIMS

1. A method of producing a cloned pig expressing a green fluorescent protein gene, comprising the steps of:

(a) preparing a nuclear donor cell by culturing a cell line collected from a pig;

(b) mixing a DNA construct carrying a green fluorescent protein (GFP) gene and a lipid component or non-lipid cationic polymer vehicle to form lipid (or cationic polymer)-DNA complexes, and adding the resulting complexes to a culture medium of the nuclear donor cell and further culturing the nuclear donor cell to introduce said GFP gene thereinto and express said GFP gene therein;

(c) transferring the transfected nuclear donor cell into an enucleated pig recipient oocyte to generate a transgenic nuclear transfer embryo, and activating said nuclear transfer embryo; and

(d) transplanting the nuclear transfer embryo into a surrogate mother pig to produce live offspring.

2. The method as set forth in claim 1, wherein the cell line collected from the pig at the step (a) is a fetal fibroblast cell.

3. The method as set forth in claim 1, wherein the DNA construct carrying the GPF gene at the step (b) is pEFGP-N1.

4. The method as set forth in claim 1, wherein the lipid component at the step (b) is FuGENE 6 or LipofectAmine Plus.

5. The method as set forth in claim 1, wherein the non-lipid cationic polymer is ExGen 500.

6. A porcine nuclear transfer embryo "SNU-P1 [Porcine NT Embryo]", which is

prepared according to the steps (a) to (c) of claim 1, and deposited at KCTC (Korean Collection for Type Cultures) under accession number KCTC 10145BP.

7. A cloned pig expressing a green fluorescent protein gene, which is produced from the porcine nuclear transfer embryo "SNU-P1 [Porcine NT Embryo]" of claim 6 by performing the step (d) of claim 1.

8. A method of producing a cloned pig having an alpha-1,3-galactosyltransferase gene knocked out, comprising the steps of:

(a) preparing a nuclear donor cell by culturing a somatic cell line collected from a pig;

(b) isolating an alpha-1,3-galactosyltransferase (GT) gene clone from a pig genomic BAC library, and constructing a gene targeting vector using the isolated GT gene, wherein the vector carries a GT gene modified by substituting a portion of a wild-type GT gene with a gene encoding a selectable marker by homologous recombination to suppress expression of a normal GT protein;

(c) mixing the vector with a lipid or non-lipid component to form lipid (or non-lipid)-DNA complexes, and adding the resulting complexes to a culture medium of the nuclear donor cell to allow gene targeting by introducing the recombinant GT gene into the nuclear donor cell;

(d) transferring the nuclear donor cells transfected with the recombinant GT gene into an enucleated pig recipient oocyte to generate a transgenic nuclear transfer embryo, and activating the nuclear transfer embryo; and

(e) transplanting the nuclear transfer embryo into a surrogate mother pig to produce live offspring.

9. The method as set forth in claim 8, wherein the cell line collected from the pig at the step (a) is a fetal fibroblast cell.

10. The method as set forth in claim 8, wherein the gene targeting vector at the step (b) is constructed not to have an exogenous promoter by a promoter trap method.

11. The method as set forth in claim 8, wherein the gene targeting vector at the step (b) comprises a nucleic acid sequence corresponding to a part of intron 8, exon 9 and a part of intron 9 of a GT gene, wherein an AvaI-DraIII fragment of said exon 9 is substituted with a nucleic acid sequence encoding a puromycin-resistant gene linked to a SV40 poly(A) sequence.

12. The method as set forth in claim 8, wherein the lipid component at the step (c) is FuGENE 6.

13. A porcine nuclear transfer embryo "SNU-P2 [Porcine NT Embryo]", which is prepared according to the steps (a) to (d) of claim 8, and deposited at KCTC (Korean Collection for Type Cultures) under accession number KCTC 10146BP.

14. A cloned pig having an alpha-1,3-galactosyltransferase gene knocked out, which is produced from the porcine nuclear transfer embryo "SNU-P2 [Porcine NT Embryo]" of claim 13 by performing the step (e) of claim 8.

15. A vector carrying a nucleic acid sequence corresponding to a part of intron 8, exon 9 and a part of intron 9 of a GT gene, wherein an AvaI-DraIII fragment of said exon 9 is substituted with a nucleic acid sequence encoding a puromycin-resistant gene linked to a SV40 poly(A) sequence.